Cells on Gels: Micron-scale polyacrylamide gels for studies of glioblastoma multiforme cell adhesion, morphology, and migration

Undergraduate Engineering Honors Research Thesis

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Abstract

Glioblastoma Multiforme (GBM) is the most commonly diagnosed brain cancer, with limited treatment options, no cure, and a median survival time of 15 months[1]. One of the reasons for GBM's poor prognosis is the cells' highly migratory behavior. GBM cells diffuse through the brain along white matter tracts creating an ill-defined tumor. Operating in the brain is risky, and with an ill-defined tumor surgery is unsuccessful at removing enough cancer cells to completely prevent recurrence. To research treatment options for GBM, there first needs to be research on the mechanisms behind GBM migration. Since migration behavior is highly dependent on substrate characteristics, the substrate needs to mimic *in vivo* conditions, meaning the substrate should have a low Young's Modulus and be patterned at the micron scale[2]. A protocol for patterning 1kPa, 10kPa, and 120kPa polyacrylamide (PA) gels with 2μm lines was established to meet this need. The protocol used a sacrificial mold made of polystyrene (PS) that can be dissolved with anisole to micropattern the PA gels consistently. GBM cells were then seeded on the PA gels and stained and imaged for analysis of cell adhesion, morphology, and migration. The PA substrates were cytocompatible and allowed for more in depth analysis of GBM migration than previous methods. This protocol could be used for analyzing individual resected tumors to provide a more accurate diagnosis for patients. The pattern on the substrate could also be changed to mimic a variety of different microenvironments within the body. The cells' initial attachment to the softer substrate was slower, which may indicate slower migration on softer substrates. This information opens up the possibility of changing the brain environment to slow migration of GBM cells.

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Introduction

Many studies have been done involving a wide variety of cancer cells, particularly cancer cells that are known for metastasizing, such as breast and pancreatic cancer[3]. These studies encompass cancers that are known to metastasize because metastasis is often the cause of a decreased survival rate in cancers. Glioblastoma multiforme (GBM) is the most commonly diagnosed brain cancer, and is highly migratory[1]. GBM diffuses however, rather than metastasizes. Diffusion is defined as cancer cell migration that stays within the primary tumor tissue. GBM cells migrate throughout the brain tissue on white matter tracts instead of extravasating. This behavior decreases survival rate of GBM patients because of the inability to resect every cancerous cell within the tissue when performing surgery on the primary tumor. If any cancer cells are left behind after resection of the primary tumor there will be recurrence of the tumor. The progression of treatment for this form of cancer is surgery, followed by radiation and chemotherapy, but the survival rate remains at about 15 months after diagnosis[1]. More research needs to be done on the diffusion of cancer cells, and on GBM diffusion in particular, to increase the survival rate. A gap in knowledge needs to be filled on the diffusion of cancer cells throughout tissue first, in order to then research treatment options.

Previous research has been done on GBM diffusion using various materials to create 2D biomimetic substrates for cell migration. One study used micropatterned polystyrene to mimic brain tissue[4]. The micropattern was similar to the white matter tracts of the brain, which GBM migrates on *in vivo*, but the Young's Modulus of the polystyrene was much

higher than these structures. These tracts have micron-scale topographical features and have a Young's Modulus of around 2kPa[2]. Polystyrene has a significantly higher Young's Modulus of 2GPa[5], which has been proven to affect cell migration. Another study used micropatterned polyacrylamide[6]. The polyacrylamide had a Young's Modulus closer to the white matter tracts *in vivo*. This study aimed to analyze the effect of channel width on cell motility. This study involved seeding GBM cells on PA channels measuring 10 and 40 microns in diameter. It was determined that channel width influenced cell polarization and migration speed. These features are far wider than the features found *in vivo,* and knowing the importance of channel width, it is necessary to develop a substrate that has microfeatures around 2μm in width. This project aimed to combine the strengths from previous research projects to create a biomimetic substrate for GBM migration analysis.

By creating a substrate that is closer to *in vivo* conditions than previous studies, more research can be done on GBM cell's migratory behavior. A new procedure was developed to create a substrate out of polyacrylamide that was patterned on the micron scale and tuned to have Young's Moduli of 1, 10, and 120kPa. The procedure is low cost, simple, and reproducible. These substrates allowed for the study of migration on substrates with various moduli, filling a gap in knowledge on GBM diffusion. This information was then used to postulate ways to decrease migration speed, such as decreasing modulus of the substrate *in vivo*. These substrates could also be used for point of care diagnostics by seeding individually resected GBM cells from patients and analyzing the speed of cell migration to give a more accurate timeline for individual patient survival.

Polyacrylamide was fabricated with a Young's Modulus of 1, 10, and 120kPa. The modulus of 1kPa was used to mimic the white matter tracts and the modulus was increased by an order of magnitude of 10 to establish the differences in cell migration velocity and morphology between the moduli. A new protocol was developed to pattern these PA gels on the micron scale that utilized a sacrificial mold made of polystyrene. The sacrificial mold allowed for easy detachment of the PA from the mold to create uniform PA gels. These gels were then seeded with U87 GBM cells and adhesion and morphology were analyzed. This research combined many different methods to develop the most accurate biomimetic substrate for GBM migration.

Methodology

Coverslip Surface Modification

15mm coverslips were treated to ensure the PA would covalently bond to the coverslip and not the mold. The coverslips were rinsed in 70% ethanol then soaked for 5 minutes in 0.1M NaOH and left to air dry. 10% silane in ethanol was then spin-coated onto the coverslips at 1000RPMs for 30s and left for 5min before being washed with DI water and left to air dry. The surface was then soaked in 0.5% glutaraldehyde in PBS for 30mn and rinsed with DI water. These coverslips were stored in PBS in a cold room set at 4 ˚C until needed for PA gel fabrication.

Sacrificial Mold Patterning

A sacrificial mold was made to pattern the PA gels. A 1:10 mixture of PDMS was made and poured over an SU8 wafer containing the desired pattern of 2um lines. The PDMS was then

poured over the wafer and degassed and put in an oven for 2 hours to harden. After cooling, the solid PDMS was cut to the size of a microscope slide and put aside. A new microscope slide was the spin-coated with 15% polystyrene in anisole at 2000RPM for 30seconds. A hot plate was heated to 175 ˚C and the slide was placed, polystyrene coating up, on the hot plate next to the PDMS mold, pattern side up. After the PS film reached temperature and the anisole evaporated, the PDMS mold was flipped and placed on the slide. A weight was placed on top after a contact line appeared in the PDMS (Figures 1 and 2) and left for 3min. The weight was then lifted from the hot plate and placed on the bench with both the PDMS and PS coated coverglass underneath for 3min to cool. The slide was peeled off the PDMS mold, resulting in a 2μm line patterned slide of polystyrene that could be used to pattern the PA and dissolved using anisole (Figure 3).

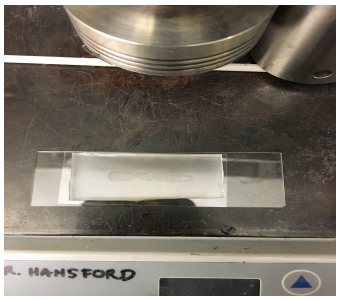

Figure 1: Patterned PDMS stamping PS on hotplate

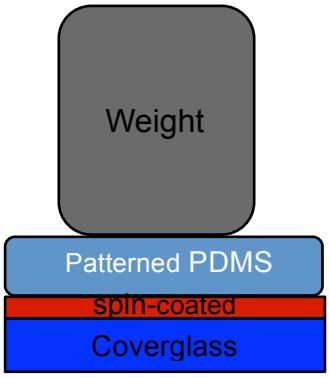

Figure 2: Fabrication of sacrificial mold using heat and Pressure to imprint pattern from PDMS

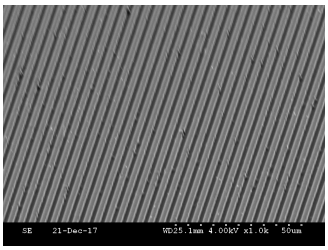

Figure 3: Micrograph of PS Pattern

Polyacrylamide Gel

Stainless steel 12mm diameter washers were taped to the patterned PS slide to ensure uniform gel diameter and height. Mixtures of acrylamide and *bis*-acrylamide were made with PBS to obtain different Young's Moduli of gels (table 1). 1000uL of this solution was

mixed with 10% APS in DI water and TEMED to initiate cross-linking. 100uL of this mixture was pipetted into the washer on the slide (figure 4). This was left to sit for an hour and a half. Anisole was then used to dissolve the polystyrene mold off the slide, which made removal of the now patterned polymerized PA gels easy. Once the gels were removed they were washed with DI water and stored in PBS until being seeded with cells.

Table 1: Polyacrylamide Ratios

Desired Modulus (kPa)	µL 40% Acrylamide	µL 2% *Bis*-Acryalmide	µL PBS
1 (5% Acrylamide, 0.03% Bis-Acrylamide)	1250	150	8550
10 (10% Acrylamide, 0.3% Bis-Acrylamide)	250	1500	5950
120 (15% Acrylamide, 1.2% Bis-Acrylamide)	3750	6000	200

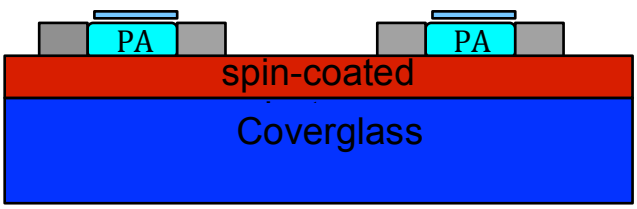

Figure 4: Fabrication of micropatterned PA Gel

Treating PA for Cell Attachment

Fibronectin (FN) was used for promoting GBM cell focal adhesions to the PA. Each polyacrylamide gel was first covered with 60µL of sulfo-SANPAH solution at a concentration 0.5mg/mL in HEPES buffer and left for 5min under UV light. Samples were exposed to 36 watts of 370nm UVA light 3 inches below the compact fluorescent UV bulbs. This solution was then removed from the gel and the process was repeated. The gels were washed 3 times in PBS, or until the PBS ran clear. 60µL of a 0.2mg/mL solution of fibronectin in PBS was then added to each gel for 2 hours. The gels were rinsed 4X with PBS and the samples were placed under PBS and stored at 4 ˚C. The sulfo-SANPAH acted as a crosslinker between the polyacrylamide and the fibronectin. The phenylazide group of the sulfo-SANPAH covalently binds to the polyacrylamide through photoactivation, leaving the sulfosuccinimidyl group exposed to react with the primary amines of the fibronectin[7].

Seeding GBM cells on Polyacrylamide

U87 cell line cells were cultured in Minimum Essential Medium Eagle with 10% fetal bovine serum and 1% penicillin. One FN treated PA gel was placed in a well of a 12-well plate with vacuum grease for stabilization. 38,000 cells were then seeded onto each gel with 1mL of PBS and 1mL of media. These gels were then imaged and stained, using immnofluorescent staining techniques, for the nucleus, focal adhesions, and actin filaments over time to determine cell migration speed, cell morphology, and cell attachment. A staining kit was used consisting of TRITC-conjugated phalloidin, anti-Vinculin, and DAPI for the focal adhesions, actin skeleton, and nucleus, respectively. The cells were then imaged after 24

and 48 hours of incubation using a fluourescent microscope. The DAPI stain was excited at 358nm and emitted 461nm fluorescence. The actin stain anti-vinculin was excited at 500nm and fluoresced at 550nm. The focal adhesion stain Phalloidin was excited at 590nm, and emits at 611nm.

Results

Cell Morphology

Cells were seeded on the PA according to the methodology outlined above. They were seeded at a number that allowed for the analysis of individual cells. After incubating for 24 hours on the PA gel, the cells were imaged in phase contrast using a light microscope.

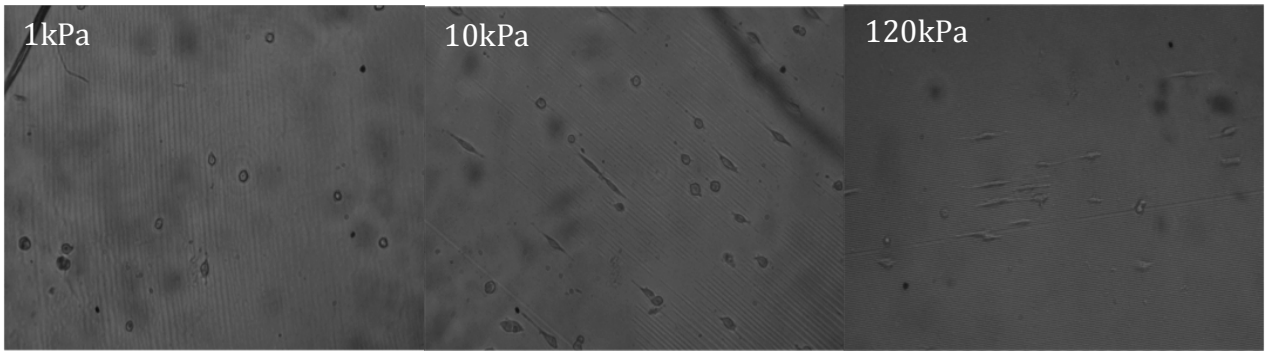

Figure 5: U87 cells on PA gels after incubating for 24 hours

The roundness of the cells was analyzed using the "fit ellipse" function in ImageJ. An ellipse was fit around 10 cells on each PA gel, shown in Figure 5. Once the ellipse was fit, the major and minor axis was measured. A ratio was used of minor axis to major axis to represent the roundness of the cell[8]. The rounder the cell, the closer this ratio would be to The roundness ratios were then uploaded to Matlab and a one-way ANOVA test was done

to determine statistical significance between the cell morphology on the gels with different Young's Moduli.

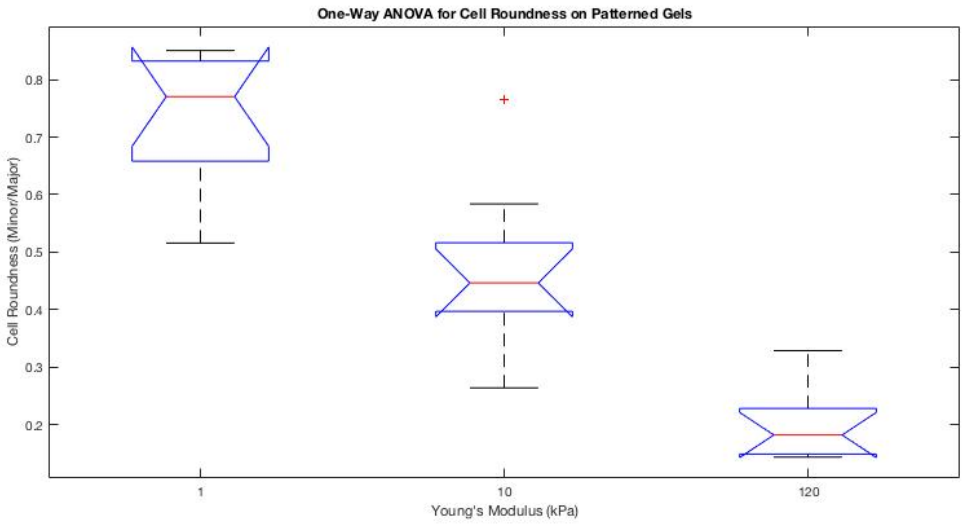

Figure 6: One-way ANOVA Test on Cell Roundness

The top black line represents the maximum ratio of the data set, the top blue line represents the 75th percentile of the data, the middle red line represents the median of the data, the bottom blue line represents the 25th percentile of the data, and the bottom black line is the minimum ratio in the data set. The red crosses are outliers in the data. The graph shows no overlap of the medians of the data sets, indicating that the true medians of each gel were statistically different with a 95% confidence level. The p-value for the data was also calculated to be 4.6579e-11, which indicates a strong statistical significance of the difference between the means of the set of data.

Cells were then imaged at various time points to determine if the cells on the 1kPa gel took longer to adhere to the substrate, or if they did not continue to elongate. These images were taken with a light microscope (phase contrast) at 48 and 72 hours. These images were from the same gels shown in Figure 5, just after increased incubation time.

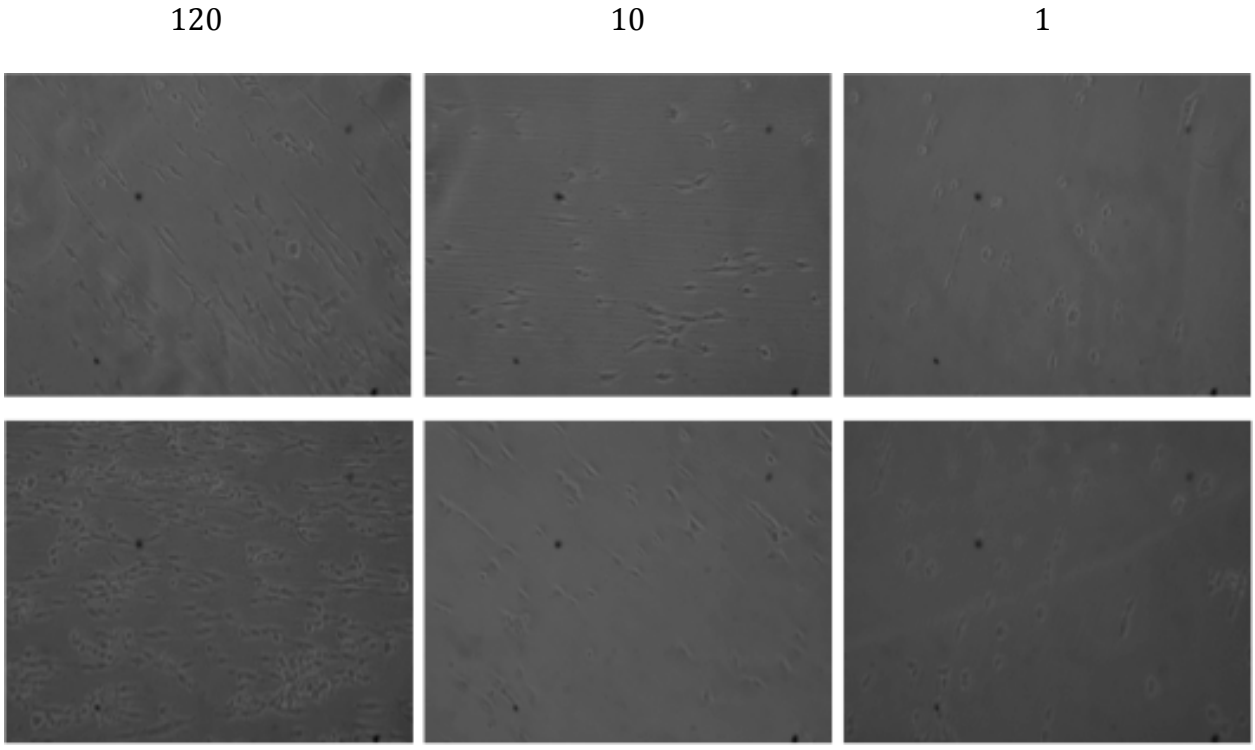

Figure 7: U87 cells on PA of 120, 10, and 1kPa at 48 (top row) and 72 hours (bottom row) incubation time

Cells were also imaged with a light microscope on flat polyacrylamide gels of the 3 different Young's Moduli. These images show the different morphology of the cells on flat versus patterned substrates to demonstrate the importance of micropatterning.

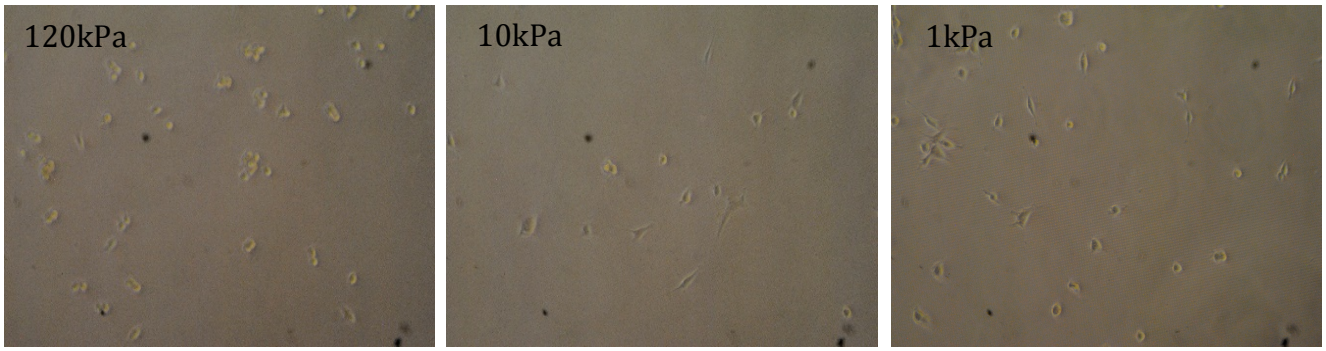

Figure 8: U87 cells seeded on flat PA gels and imaged on light microscope at 24 hours incubation time

Statistical analysis was done to determine if there was a difference in cell morphology for the difference in Young's Modulus without the pattern. The reported p-value was 0.0325, which indicates statistical significance, but not as strong of a correlation as when the PA gels were micropatterned.

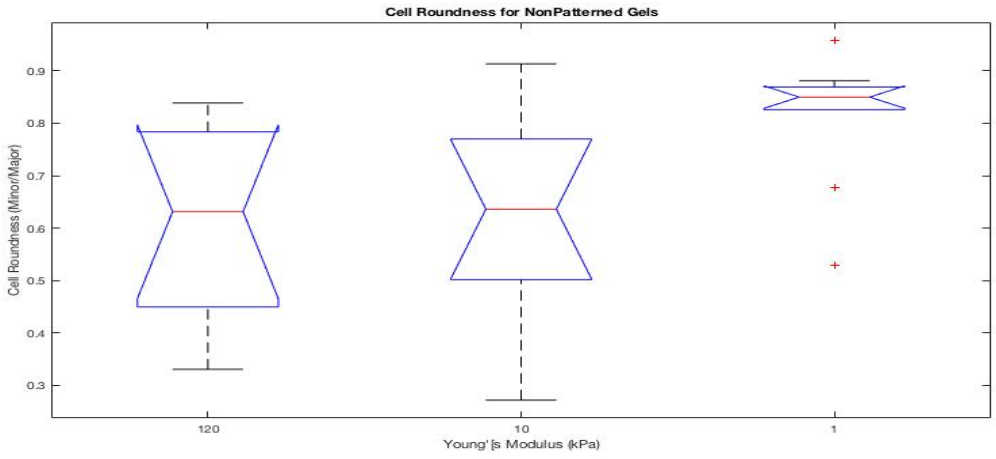

Figure 9: one-way ANOVA Test on Cell Roundness on Flat PA Gels

Cells were then seeded on polystyrene to compare cell morphology on a patterned substrate that had a significantly higher Young's Modulus (4GPa) than the polyacrylamide

to confirm that Young's Modulus influences cell behavior. This was done by seeding U87 cells onto the PS mold that was creating using the protocol above for PS patterning from PDMS stamping. The detailed protocol for this is in the Appendix.

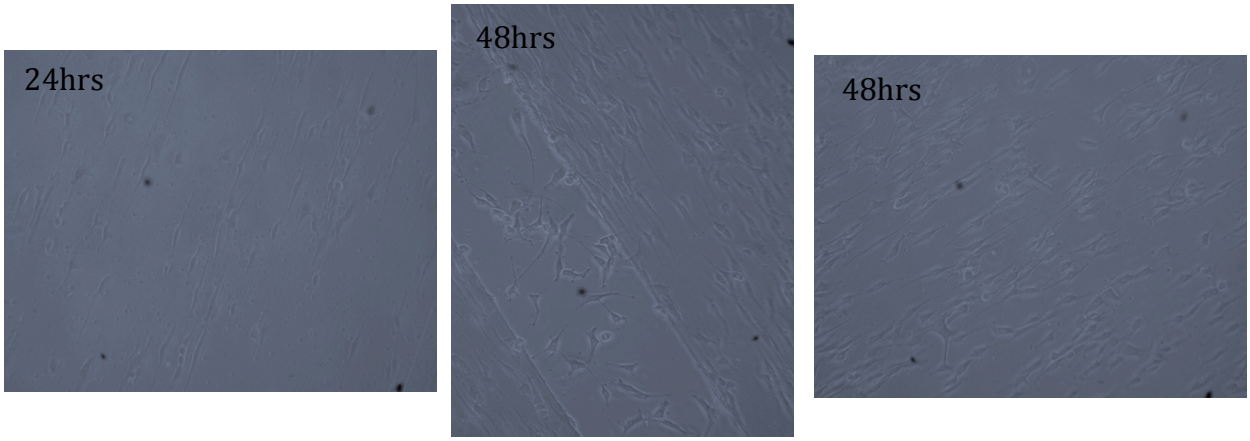

Figure 10: U87 Cells Seeded on Patterned Polystyrene at 24 and 48 hours

These images show enhanced elongation compared to the PA gels. The middle image also shows the alignment on the patterned parts of the substrate compared to the non-patterned substrate, further supporting the need for a substrate that has a low Young's Modulus and is patterned on the micron scale.

Cell Staining

Cells were then stained for the actin skeleton (green), focal adhesions (red), and nuclei (blue) to further analyze the differences of cell morphology based on the modulus of the substrate.

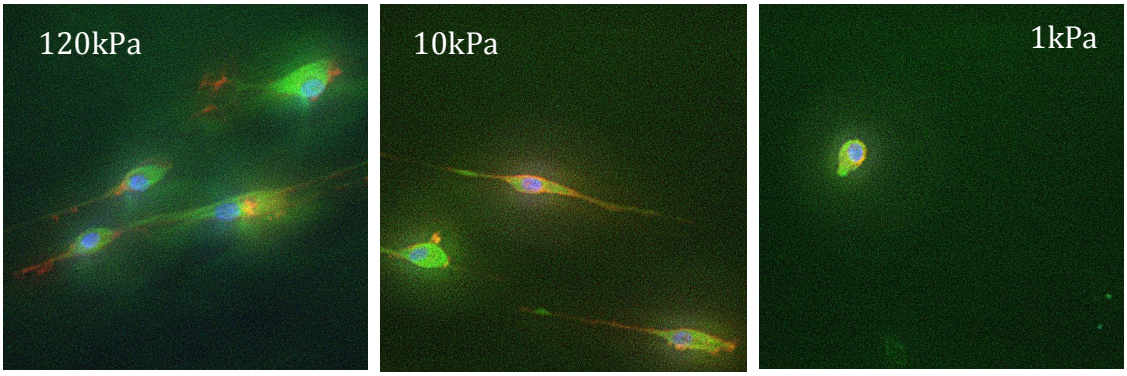

Figure 11: Fluorescent micrographs of cells on patterned PA gels after 12 hrs

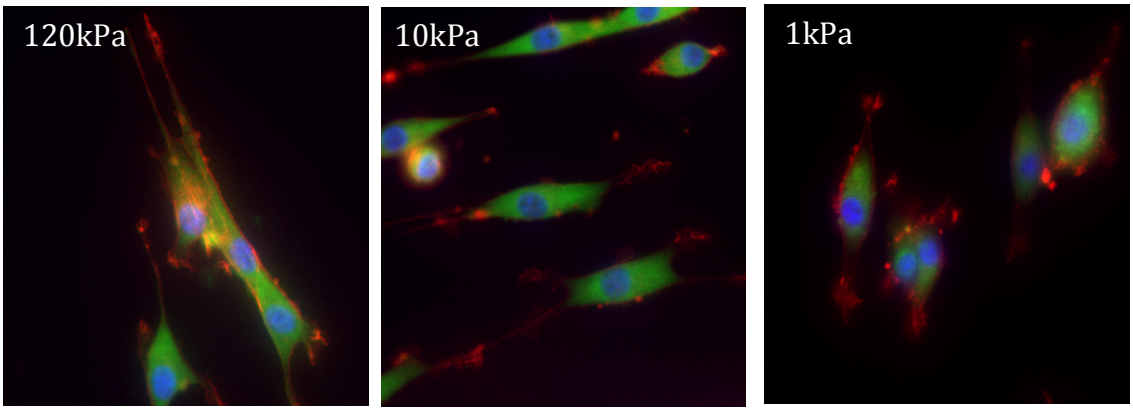

Figure 12: Fluorescent micrographs of cells on patterned PA gels after 36 hrs

These images show the spread of the actin skeleton and where the focal adhesions are attaching to the substrate. This actin is polymerized within the cell to create the lammellipodia, which is connected to the substrate via focal adhesions. The actin creates tension between focal adhesions, which is utilized when the trailing edge of the cell detaches from the substrate, allowing for migration. These images show increased actin and focal adhesion spread on the gels with higher Young's Moduli. This indicates increased

tension build-up in these cells. These images also show the differences in the morphology over time. The actin skeleton appears to spread out more as incubation time increases.

Discussion

Polyacrylamide gel fabrication

Polyacrylamide gels with 2μm lines were fabricated consistently. The ratios of *bis*-acrylamide to acrylamide were selected to give Young's Moduli of 1kPa, 10kPa, and 120kPa. In previous papers DI water was mixed with these ratios, but PBS was used in this methodology. The PBS created less hygroscopic PA gels, which decreased the swelling of the gels that took place between fabrication and use. Using PBS in the mixture, and then storing the gels in PBS instead of DI water preserved the microfeatures on the gel. The microfeatures remained straight instead of wavy, which was previously caused by swelling with gels produced from DI water. Stainless steel washers were taped to the PS pattern before PA was pipetted onto the pattern. These washers ensured that all the gels had a uniform diameter and thickness. This consistency in the gel size was used to accurately calculate and seed the same amount of cells onto the gels throughout the experiments. The gels were also patterned consistently using a sacrificial mold of PS. Previous methods involved pipetting the PA mixture onto the micropatterned silicon wafer and placing the treated coverslip on top and then peeling the coverslip and PA off of the wafer. This method worked for stiffer substrates, but proved difficult with the 1kPa gel. The gel was sticking to the wafer, causing damage to the wafer and variation in the pattern on the PA gel. To circumvent this problem a PDMS mold was made from the wafer and then used to

stamp PS in anisole. This transferred the pattern to a mold that could be dissolved using anisole. Dissolving the mold meant there was no need to peel the gel from the pattern, thus creating uniformly patterned PA gels. The PDMS could also be reused as a stamp, so the process of creating this PS mold was cheap and efficient. This technique was used throughout the experiment to gain information about GBM cell adhesion, morphology, and migration.

Cell Morphology and Adhesion

Initial images were taken of the GBM cells using a light microscope on three gels of the three different Young's Moduli. The images taken at 24 hours show varying cell morphology between the three gels with three different Young's Moduli. The cells on the 1kPa gel were more circular than the cells on the 10kPa or 120kPa. The cells appeared the most elongated on the 120kPa gel. This was further proven by performing a one-way ANOVA test to determine if there was a significant difference in the means of the cell roundness. Cell roundness was calculated by using the fit ellipse function in ImageJ to get a major and minor axis measurement. A ratio of minor to major axis was then used to determine roundness. Values closer to 1 meant that the major and minor axis were closer together, meaning the cell was rounder. The graphs shown in Figure 5 show that the medians were statistically different. The low p-value suggests that with 95% confidence the cell roundness average was statistically different based on gel modulus. The increased elongation could be a sign of faster adhesion to substrates with larger Young's Moduli, which should lead to increased migration speed. This would be expected as the larger the Young's Modulus; the more stress the material could withstand and still return to its normal shape. A cell moves by first creating a protruding edge, which attaches to the

substrate. This creates tension within the cell and the trailing edge detaches from the substrate, causing the cell to propel forward. Higher degrees of elongation suggest more tension build up in the actin skeleton of the cell, and longer distances travelled with one cell movement cycle than a cell that is rounder. Referring to the model for cell migration, it would be apparent that a more elastic substrate would lead to more tension buildup in the cell to propel it forward. It has been shown in previous studies that substrates with higher Young's Modulus incite higher cell migration speeds. This was explored further by taking images at further time points. These images prove that the larger the Young's Moduli, the more elongated the cells. The cells on the 1kPa gel elongate further as time goes on, but do not reach the elongation seen on the stiffer substrates. The cells were then stained for the actin skeleton, focal adhesions, and nucleus. These images show a more spread out actin skeleton and focal adhesions farther from the nucleus when the modulus is higher. This indicates that the cell is elongating more and attaching to the substrate farther from the center of the cell. Increased elongation of the actin skeleton creates increased tension within the cell. The cell utilizes this tension to propel the cell foreword. Therefore, the cells exhibiting more actin elongation will migrate faster than cells exhibiting a more rounded morphology. The cells on the 1kPa gels exhibit the rounder morphology. Focal adhesions are seen on the rounder cells on the 1kPa gel, indicating that they are attempting to migrate along the substrate. The cell cannot create the same tension due to the decreased elasticity of the gel. For the cell to move on this less elastic substrate the cell deforms the gel more extensively in attempting to create the tension needed for cell movement, resulting in slower and less effective migration. The stained images taken at 24, then 48 hours also indicates that the cells on the 1kPa gel take longer to elongate than

the other cells, but they still do not reach the extent of elongation seen on the other gels. This further suggests faster migration of GBM cells on substrates with higher Young's Modulus. It is known that tumor stiffness is higher than surrounding tissue due to tumor cells altering the composition and physical properties of the extracellular matrix[9]. This research indicates that this increased stiffness leads to enhanced cell migration. This increased cell migration means increased GBM diffusion throughout the brain tissue, making the cancer extremely difficult to treat or even control. Now that this migration is better understood future work should include research on decreasing GBM cell migration to enhance current treatment techniques and create new ones.

Conclusion

In conclusion, there is a drastic need for a substrate that mimics *in vivo* conditions to study Glioblastoma Multiforme migration more extensively. This protocol consistently provided patterned polyacrylamide with varying Young's Modulus. These substrates were then seeded with U87 cells, and cell adhesion and morphology analysis was done to establish differences between the gels of various moduli. It was determined that there was a significant difference in cell adhesion and morphology between the various PA gels. Cells adhered quicker and elongated more extensively on gels with the highest Young's Modulus of 120kPa. This indicates more rapid migration of GBM cells on stiffer substrates. Based on this knowledge, a strategy for softening the substrate *in vivo* could slow down GBM cell diffusion, and increase patient survivability. This also suggests that increased tumor stiffness, due to cancer cell remodeling of the extracellular matrix, leads to increase cell

migration. Live imaging will be done in the future to calculate cell velocity, and is hypothesized that the higher the modulus the faster the cells will migrate.

Future Studies

Future studies will include live image analysis to determine velocity of cell migration on each gel. It is hypothesized that this analysis will show increased migration speeds on substrates with higher moduli, based on the images taken in the previous experiments. Biomarkers could also be determined for increased migration speeds of GBM using the 1kPa substrate that closely mimics the environment *in vivo.* The biomarkers could be utilized in a clinical setting to identify patients with more or less aggressive forms of GBM to give a more accurate diagnosis. Patient derived cells could be seeded onto the substrate and analyzed using the known biomarkers to determine how aggressive the patient specific tumor is. In broader applications, this technique for patterning polyacrylamide could be used with a wide variety of patterns created on silicon wafers, and be used to study cell migration of other types of cancers and diseases in general. These substrates are also being studied in conjunction with microfluidic devices to create 3D microfluidic devices with more *in vivo* like conditions. There are multiple uses for this methodology due to its low cost and high efficiency. These attributes lend to its applicability in a clinical setting, which would be the ultimate goal.

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Appendices

Chart of Cell Roundness Data

120kPa	Area	Mean	Major	Minor	Angle	Cell Circularity Ratio: Major/Minor	Cell Circularity: Minor/Major
1	132	54.697	34.241	4.908	3.724	6.976568867	0.143336935
2	90	54.089	22.424	5.11	19.539	4.388258317	0.227880842
3	103	55.078	27.874	4.705	5.176	5.924335813	0.168795293
4	97	58.701	28.831	4.284	9.574	6.729925303	0.148590059
5	105	57.048	26.74	5	8.435	5.348	0.186985789
6	83	58.928	24.466	4.319	4.192	5.664737208	0.176530696
7	128	60.141	27.399	5.948	10.396	4.606422327	0.217088215
8	69	61.696	17.836	4.926	5.475	3.620787657	0.276183001
9	108	59.296	30.743	4.473	5.458	6.873015873	0.145496536
10	106	60.462	20.266	6.66	5.469	3.042942943	0.328629231
1kPa							
1	*29*	*59.862*	*6.883*	*5.364*	*90*	1.283184191	0.779311347
2	*28*	*48.643*	*7.372*	*4.836*	*157.226*	1.524400331	0.655995659
3	*42*	*47.071*	*8.182*	*6.536*	*136.949*	1.251835985	0.798826693
4	*62*	*55.79*	*9.737*	*8.107*	*69.542*	1.201060812	0.832597309
5	*35*	*55.6*	*7.829*	*5.692*	*90.858*	1.375439213	0.72704049
6	*35*	*54.086*	*8.227*	*5.416*	*9.247*	1.519017725	0.658320165

7	*59*	*49.068*	*12.067*	*6.225*	*88.21*	1.938473896	0.515869727
8	*43*	*45.256*	*8.478*	*6.458*	*92.884*	1.312790338	0.761736259
9	*53*	*44.491*	*8.931*	*7.556*	*157.559*	1.18197459	0.846041877
10	108	39.38	12.712	10.817	164.101	1.175187205	0.850928257
10kPa							
1	*80*	*52.988*	*16.938*	*6.014*	*147.007*	2.816428334	0.355059629
2	*84*	*50.845*	*13.532*	*7.903*	*121.296*	1.712261167	0.584023056
3	*47*	*47.213*	*12.28*	*4.873*	*153.901*	2.520008208	0.396824104
4	*58*	*46.034*	*13.13*	*5.624*	*137.183*	2.334637269	0.428332064
5	*50*	*48.72*	*12.077*	*5.271*	*138.471*	2.291216088	0.436449449
6	*56*	*43.929*	*11.752*	*6.067*	*134.622*	1.937036427	0.516252553
7	*84*	*47.19*	*15.307*	*6.987*	*148.905*	2.190782882	0.45645783
8	*69*	*56.232*	*10.708*	*8.205*	*147.994*	1.305057892	0.766249533
9	*80*	*50.3*	*19.652*	*5.183*	*147.949*	3.791626471	0.26373906
10	68	49.632	13.308	6.506	157.895	2.045496465	0.48887887
1 Nonpat terned							
1	67	90.179	10.018	8.515	156.271	1.176512038	0.849970054
2	104	94.933	12.381	10.695	20.534	1.157643759	0.863823601
3	46	97.109	10.533	5.561	88.938	1.894083798	0.527959746
4	23	99.913	5.884	4.977	59.957	1.182238296	0.845853161

5	74	89.986	10.411	9.05	56.524	1.15038674	0.869272884
6	67	83.612	10.163	8.394	127.142	1.210745771	0.825937223
7	44	86.75	7.645	7.328	146.31	1.043258734	0.95853499
8	52	87.712	8.825	7.502	164.991	1.176352973	0.850084986
9	42	88.524	7.789	6.865	50.52	1.134595776	0.881371164
10	77	85.182	12.027	8.152	50.309	1.475343474	0.677808265
10 non							
1	61	92.656	14.202	5.469	77.575	2.596818431	0.385086608
2	77	90.987	10.36	9.463	127.459	1.094790236	0.913416988
3	50	95.34	8.598	7.404	123.324	1.161264182	0.861130495
4	58	82.431	11.16	6.617	71.52	1.686564909	0.592921147
5	63	82.254	10.207	7.859	109.57	1.298765746	0.769961791
6	103	98.913	13.896	9.437	125.181	1.472501854	0.679116292
7	67	94.448	12.043	7.084	83.821	1.700028233	0.588225525
8	179	88.536	17.441	13.068	49.057	1.334634221	0.749268964
9	72	85.792	13.522	6.78	33.497	1.99439528	0.501405118
10	91	87.626	20.66	5.608	40.979	3.684022825	0.271442401
120 non							
1	78	93.795	12.962	7.662	83.005	1.691725398	0.591112483
2	54	88.519	14.429	4.765	111.442	3.028121721	0.330237716

3	63	90.714	10.931	7.338	86.265	1.489642954	0.671301802
4	73	86.808	12.779	7.273	104.882	1.757046611	0.569136865
5	66	82.924	13.678	6.144	75.111	2.226236979	0.449188478
6	37	93	7.494	6.286	156.773	1.192173083	0.838804377
7	70	84.571	10.665	8.357	43.087	1.276175661	0.783591186
8	57	85.439	9.306	7.798	60.871	1.193382919	0.837954008
9	79	80.62	11.6	8.671	78.479	1.337792642	0.7475
10	127	91.425	19.127	8.454	148.338	2.2624793	0.441992994

Matlab Script for one-way ANOVA on Patterned Gels (Used for all ANOVA analysis)

```
clc
clear all

y120=[0.143336935
0.227880842
0.168795293
0.148590059
0.186985789
0.176530696
0.217088215
0.276183001
0.145496536
0.328629231];

y10=[0.355059629
0.584023056
0.396824104
0.428332064
0.436449449
0.516252553
0.45645783
0.766249533
0.26373906
0.48887887];

y1=[0.779311347
0.655995659
0.798826693
```

0.832597309
0.72704049
0.658320165
0.515869727
0.761736259
0.846041877
0.850928257];

Y=[y120(:,1),y10(:,1),y1(:,1)];

[p,tbl,stats] = anova1(Y)

Full ANOVA Results for Patterned Gels

Source	SS	df	MS	F	Prob>F
Columns	1.46193	2	0.73096	65.14	4.65791e-11
Error	0.30297	27	0.01122		
Total	1.7649	29			

Full ANOVA Results for Non-Patterned Gels

Source	SS	df	MS	F	Prob>F
Columns	0.23182	2	0.11591	3.9	0.0325
Error	0.80214	27	0.02971		
Total	1.03396	29			